Published in final edited form as:
Pediatr Blood Cancer. 2009 September ; 53(3): 509–512. doi:10.1002/pbc.21976.

Initial Testing of Aplidin by the Pediatric Preclinical Testing Program

Christopher L. Morton, BS[1], **Peter J. Houghton, PhD**[1], **Richard Gorlick, MD**[2], **E. Anders Kolb, MD**[3], **Richard Lock, PhD**[4], **Hernan Carol, PhD**[4], **Stephen T. Keir, PhD**[5], **C. Patrick Reynolds, MD, PhD**[6], **Min H. Kang, PharmD**[6], **John M. Maris, MD**[7], **Catherine Billups, MS**[1], and **Malcolm A. Smith, MD, PhD**[8]

[1] St. Jude Children's Research Hospital, Memphis, TN

[2] The Children's Hospital at Montefiore, Bronx, NY

[3] A.I. duPont Hospital for Children, Wilmington, DE

[4] Children's Cancer Institute Australia for Medical Research, Randwick, NSW, Australia

[5] Duke University Medical Center, Durham, NC

[6] Texas Tech University Health Sciences Center, Lubbock, TX

[7] Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine and Abramson Family Cancer Research Institute, Philadelphia, PA

[8] Cancer Therapy Evaluation Program, NCI, Bethesda, MD

Abstract

Aplidin was tested *in vitro* at concentrations ranging from from 0.1 nM to 1.0 μM and *in vivo* at a dose of 0.6 mg/kg administered intraperitoneally on an every 4 days × 3 schedule that was repeated at day 21. *In vitro,* Aplidin was most active against acute lymphoblastic leukemia (ALL) cell lines. *In vivo,* Aplidin induced significant differences in EFS distribution in 12 of 28 (43%) solid tumor models and 2 of 6 evaluable ALL models. Aplidin showed potent *in vitro* activity and induced significant *in vivo* tumor growth inhibition in some xenografts, but did not induce tumor regressions.

Keywords

Preclinical Testing; Developmental Therapeutics; Aplidin

INTRODUCTION

Aplidin (Plitidepsin), a potent antitumor agent, was first isolated from the marine tunicate *Aplidium albacans* and currently is obtained by total chemical synthesis. The mechanism of action of Aplidin is not clearly defined, although apoptosis induction has been described as occurring through a strong, sustained activation of c-Jun NH2-terminal kinase (JNK) [1]. Cells resistant to Aplidin show lesser extent of JNK activation [2]. JNK activation in human breast cancer cells was reported to be dependent upon induction of oxidative stress associated with activation of the Rac1 small GTPase [3]. Aplidin is currently in Phase II

Corresponding Author: Peter J. Houghton, PhD, Department of Molecular Pharmacology, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105, Voice: (901) 595-3440, Fax: (901) 595-4290, peter.houghton@stjude.org.

clinical evaluation as a single agent for solid and hematologic malignant neoplasias including multiple myeloma, non-Hodgkin lymphoma, and acute lymphoblastic leukemia (ALL). Phase I studies in pediatric acute leukemias and solid tumors as well as combination studies with other chemotherapeutic drugs are currently ongoing. Given the potential novel mechanism of action of Aplidin, the PPTP evaluated this agent to gain insight into its utility against pediatric tumors.

MATERIALS AND METHODS

In vitro testing

In vitro testing was performed using DIMSCAN, a semiautomatic fluorescence-based digital image microscopy system that quantifies viable cell numbers in tissue culture multiwell plates [4]. Cells were incubated in the presence of Aplidin for 96 hours at concentrations from 0.1 nM to 1.0 μM and analyzed as previously described [5].

In vivo tumor growth inhibition studies

CB17SC-M *scid*$^{-/-}$ female mice (Taconic Farms, Germantown NY), were used to propagate subcutaneously implanted kidney/rhabdoid tumors, sarcomas (Ewing, osteosarcoma, rhabdomyosarcoma), neuroblastoma, and non-glioblastoma brain tumors, while BALB/c nu/nu mice were used for glioma models, as previously described [6–8]. Human leukemia cells were propagated by intravenous inoculation in female non-obese diabetic (NOD)/*scid*$^{-/-}$ mice as described previously [9]. All mice were maintained under barrier conditions and experiments were conducted using protocols and conditions approved by the institutional animal care and use committee of the appropriate consortium member. Tumor volumes (cm^3) [solid tumor xenografts] or percentages of human CD45-positive [hCD45] cells [ALL xenografts] were determined as previously described [10]. Responses were determined using three activity measures as previously described [10]. An in-depth description of the analysis methods is included in the Supplemental Response Definitions section.

Statistical Methods

The exact log-rank test, as implemented using Proc StatXact for SAS®, was used to compare event-free survival distributions between treatment and control groups. P-values were two-sided and were not adjusted for multiple comparisons given the exploratory nature of the studies.

Drugs and Formulation

Aplidin was provided to the PPTP by PharmaMar, through the Cancer Therapy Evaluation Program (NCI). Aplidin was reconstituted in a solution of Cremophor: Ethanol: Water (15:15:70) and further diluted in saline. Aplidin was administered i.p., every 4 days times 3, repeated at day 21, at a dose of 0.6 mg/kg. Aplidin was provided to each consortium investigator in coded vials for blinded testing.

RESULTS

Aplidin in vitro testing

Aplidin demonstrated a high level of cytotoxic activity against the PPTP's cell lines, with 16 of 23 cell lines showing T/C values < 50% at the lowest Aplidin concentration tested (0.1 nM)(Table I). Therefore, Aplidin cytotoxic activity at 0.1 nM ($T/C_{0.1nM}$) was used to compare the relative responsiveness of the PPTP cell lines to Aplidin. The median $T/C_{0.1nM}$ was 25.3% (range 0.5% to 96.3%). The cell lines of the ALL panel were the most sensitive PPTP cell lines to Aplidin (median $T/C_{0.1nM}$ = 8.6%), whereas the neuroblastoma ($T/C_{0.1nM}$

= 80.8%) and rhabdomyosarcoma ($T/C_{0.1nM}$ = 57.4%) cell lines were less responsive to Aplidin (Table I and Supplemental Figure 1).

Activity of Aplidin against the PPTP in vivo panel

Aplidin was evaluated in 44 xenograft models. Fifty-nine of 830 mice died during the study (7.1%), with 3 of 411 in the control arms (0.7%) and 56 of 419 in the Aplidin treatment arms (13.4%). Ten lines were excluded from analysis due to toxicity greater than 25 percent. A complete summary of results is provided in Supplemental Table I, including total numbers of mice, number of mice that died (or were otherwise excluded), numbers of mice with events and average times to event, tumor growth delay, as well as numbers of responses and T/C values.

Antitumor effects were evaluated using the PPTP activity measures for time to event (EFS T/C), tumor growth delay (tumor volume T/C), and objective response. Aplidin induced significant differences in EFS distributions compared to controls in 12/28 (43%) of the evaluable solid tumor models and 2 of 6 (33%) for the evaluable ALL xenografts (Table II). Only two lines (ALL-3, ALL-8) met the criteria for intermediate activity with EFS T/C values of 11.2 and 4.7, respectively (Table II). No other models met criteria for intermediate activity for the EFS T/C activity measure by having EFS T/C values exceeding 2.0.

The *in vivo* testing results for the objective response measure of activity are presented in Supplemental Figure 2 in a ‘heat-map’ format as well as a ‘COMPARE’-like format, based on the scoring criteria described in the Material and Methods and the Supplemental Response Definitions section. The latter analysis demonstrates relative tumor sensitivities around the midpoint score of 5 (stable disease). Objective responses were seen in 0 of 34 tumor models.

DISCUSSION

In vitro the ALL cell lines appeared more sensitive to Aplidin compared to other lines, in agreement with previous reports of Aplidin activity against ALL cell lines and primary ALL cells [11,12]. By contrast, the neuroblastoma and rhabdomyosarcoma cell lines appeared less responsive to Aplidin than the remaining cell lines. However, relative sensitivities using IC_{50} concentrations cannot be determined from our analysis.

Aplidin demonstrated limited activity against the *in vivo* solid tumor panels. While there were clearly treatment effects as demonstrated by significant differences in EFS distribution between treated and control animals, these effects were modest and in no case did the time to event for a treated group exceed that for a control group by a factor of two or greater. Aplidin showed greater activity against the PPTP’s ALL panel compared to the solid tumor panels, although the level of activity observed for Aplidin was less than that previously noted for standard agents (e.g., vincristine and cyclophosphamide) [10]. Two xenografts (ALL-3 and ALL-8) had significant extensions in time to event, but they did not meet criteria for objective response.

One important issue in assessing the PPTP *in vivo* data is whether the systemic exposures achieved in test animals are comparable to systemic exposures achieved in humans at tolerable doses. The estimated plasma systemic exposure in mice for the planned six week treatment course is approximately 1000 ng*hr/mL (personal communication[1]). For humans, the recommended phase II dose for Aplidin using an every two week schedule is 5–7 mg/m^2 [13]. The estimated plasma systemic exposure for a six week treatment course at the 5 mg/

[1]Doreen LePage, PharmaMar.

m^2 dose level is approximately 1050 ng*hr/mL, which is similar to the estimated exposure in mice.

The PPTP Stage 1 testing results point to ALL as the diagnosis for which Aplidin has the highest level of activity, though this activity does not equal that of standard agents previously studied. Given the PPTP results as well as prior published results describing anti-leukemia activity for Aplidin, consideration should be given to focusing future research efforts on optimizing the activity of Aplidin (either used alone or in combination) against ALL.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NO1-CM-42216, CA21765, and CA108786 from the National Cancer Institute and used Aplidin supplied by PharmaMar. In addition to the authors represents work contributed by the following: Sherry Ansher, Ingrid Boehm, Joshua Courtright, Mila Dolotin, Edward Favours, Henry S. Friedman, Min Kang, Debbie Payne-Turner, Charles Stopford, Chandra Tucker, Amy E. Watkins, Jianrong Wu, Joe Zeidner, Ellen Zhang, and Jian Zhang. Children's Cancer Institute Australia for Medical Research is affiliated with the University of New South Wales and Sydney Children's Hospital.

References

1. Cuadrado A, Gonzalez L, Suarez Y, et al. JNK activation is critical for Aplidin-induced apoptosis. Oncogene. 2004; 23(27):4673–4680. [PubMed: 15122339]
2. Losada A, Lopez-Oliva JM, Sanchez-Puelles JM, et al. Establishment and characterisation of a human carcinoma cell line with acquired resistance to Aplidin. Br J Cancer. 2004; 91(7):1405–1413. [PubMed: 15365569]
3. Gonzalez-Santiago L, Suarez Y, Zarich N, et al. Aplidin induces JNK-dependent apoptosis in human breast cancer cells via alteration of glutathione homeostasis, Rac1 GTPase activation, and MKP-1 phosphatase downregulation. Cell Death Differ. 2006; 13(11):1968–1981. [PubMed: 16543941]
4. Frgala T, Kalous O, Proffitt RT, et al. A fluorescence microplate cytotoxicity assay with a 4-log dynamic range that identifies synergistic drug combinations. Mol Cancer Ther. 2007; 6(3):886–897. [PubMed: 17363483]
5. Houghton PJ, Morton CL, Kolb EA, et al. Initial testing (stage 1) of the proteasome inhibitor bortezomib by the pediatric preclinical testing program. Pediatr Blood Cancer. 2008; 50(1):37–45. [PubMed: 17420992]
6. Friedman HS, Colvin OM, Skapek SX, et al. Experimental chemotherapy of human medulloblastoma cell lines and transplantable xenografts with bifunctional alkylating agents. Cancer Res. 1988; 48(15):4189–4195. [PubMed: 3390813]
7. Graham C, Tucker C, Creech J, et al. Evaluation of the antitumor efficacy, pharmacokinetics, and pharmacodynamics of the histone deacetylase inhibitor depsipeptide in childhood cancer models in vivo. Clin Cancer Res. 2006; 12(1):223–234. [PubMed: 16397046]
8. Peterson JK, Tucker C, Favours E, et al. In vivo evaluation of ixabepilone (BMS247550), a novel epothilone B derivative, against pediatric cancer models. Clin Cancer Res. 2005; 11(19 Pt 1):6950–6958. [PubMed: 16203787]
9. Liem NL, Papa RA, Milross CG, et al. Characterization of childhood acute lymphoblastic leukemia xenograft models for the preclinical evaluation of new therapies. Blood. 2004; 103(10):3905–3914. [PubMed: 14764536]
10. Houghton PJ, Morton CL, Tucker C, et al. The pediatric preclinical testing program: description of models and early testing results. Pediatr Blood Cancer. 2007; 49(7):928–940. [PubMed: 17066459]

11. Bresters D, Broekhuizen AJ, Kaaijk P, et al. In vitro cytotoxicity of aplidin and crossresistance with other cytotoxic drugs in childhood leukemic and normal bone marrow and blood samples: a rational basis for clinical development. Leukemia. 2003; 17(7):1338–1343. [PubMed: 12835722]

12. Erba E, Serafini M, Gaipa G, et al. Effect of Aplidin in acute lymphoblastic leukaemia cells. Br J Cancer. 2003; 89(4):763–773. [PubMed: 12915891]

13. Faivre S, Chieze S, Delbaldo C, et al. Phase I and pharmacokinetic study of aplidine, a new marine cyclodepsipeptide in patients with advanced malignancies. J Clin Oncol. 2005; 23(31):7871–7880. [PubMed: 16172454]

Table I

Activity of Aplidin against Cell Lines in the PPTP *in Vitro* Panel

Cell Line	Status	Histology	$T/C_{0.1nM}$ (%)	IC_{50} (nM)
RD		Rhabdomyosarcoma	46.4	<0.1
Rh41	Post-Therapy	Rhabdomyosarcoma	57.5	0.19
Rh18	Diagnosis	Rhabdomyosarcoma	96.3	5.50
Rh30	Diagnosis	Rhabdomyosarcoma	57.3	0.23
BT-12	Diagnosis	Rhabdoid	24.8	<0.1
CHLA-266	Diagnosis	Rhabdoid	35.7	<0.1
TC-71	Post-Therapy	Ewing	5.5	<0.1
CHLA-9	Diagnosis	Ewing	32.9	<0.1
CHLA-10	Post-Therapy	Ewing	25.3	<0.1
CHLA-258	Post-Bone Marrow Transplant	Ewing	46.7	<0.1
SJ-GBM2	Post-Therapy	Glioblastoma	15.5	<0.1
NB-1643	Diagnosis	Neuroblastoma	79.5	0.67
NB-EBc1	Post-Therapy	Neuroblastoma	95.4	5.70
CHLA-90	Post-Bone Marrow Transplant	Neuroblastoma	35.0	<0.1
CHLA-136	Post-Bone Marrow Transplant	Neuroblastoma	82.1	6.00
COG-LL-317	Post-Therapy	ALL T-cell	3.5	<0.1
NALM-6	Post-Therapy	ALL B-precursor	0.5	<0.1
RS4;11	Post-Therapy	ALL B-precursor	21.6	<0.1
MOLT-4	Post-Therapy	ALL T-cell	8.6	<0.1
CCRF-CEM		ALL T-cell	11.0	<0.1
Kasumi-1	Post-Bone Marrow Transplant	AML	11.4	<0.1
Karpas-299	Post-Therapy	ALCL	8.8	<0.1
Ramos-RA1		NHL	3.7	<0.1
Median			**25.3**	**<0.1**
Minimum			**0.5**	**<0.1**
Maximum			**96.3**	**6.00**

Table II

Activity for Aplidin against the PPTP *in Vivo* Panel

Xenograft Line	Histology	P-value	EFS T/C	Median Final RTV	Tumor Volume T/C	P-value	T/C Activity	EFS Activity	Response Activity
BT-29	Rhabdoid	**0.009**	1.4	>4	0.52	0.002	Low	Low	Low
KT-14	Rhabdoid	0.33	1.5	>4	0.9	0.36	Low	Low	Low
KT-12	Rhabdoid	0.406	1.1	>4	0.82	0.274	Low	Low	Low
KT-10	Wilms	0.259	1.2	>4	0.64	0.043	Low	Low	Low
KT-11	Wilms	0.104	1.3	>4	0.71	0.105	Low	Low	Low
KT-13	Wilms	**0.048**	1.2	>4	0.83	0.113	Low	Low	Low
SK-NEP-1	Ewing	0.224	1.2	>4	0.82	0.19	Low	Low	Low
CHLA258	Ewing	0.825	1	>4	0.79	0.631	Low	Low	Low
Rh28	ALV RMS	0.831	1.3	>4	0.75	0.573	Low	Low	Low
Rh30	ALV RMS	0.19	0.8	>4	1.03	0.971	Low	Low	Low
Rh30R	ALV RMS	0.26	1.1	>4	0.9	0.258	Low	Low	Low
Rh41	ALV RMS	**0.025**	1.1	>4	0.79	0.094	Low	Low	Low
Rh65	ALV RMS	0.16	1.4	>4	0.86	0.631	Low	Low	Low
Rh18	EMB RMS	**0.002**	1.5	>4	0.58	0.001	Low	Low	Int
BT-45	Medulloblastoma	**0.033**	1.4	>4	0.76	0.237	Low	Low	Low
BT-41	Ependymoma	0.549	0.9	>4	0.97	0.867	Low	Low	Low
GBM2	Glioblastoma	<**0.001**	1.5	>4	0.67	0.019	Low	Low	Low
D456	Glioblastoma	0.106	1.2	>4	0.79	0.093	Low	Low	Low
NB-SD	Neuroblastoma	**0.007**	1.4	>4	0.53	<0.001	Low	Low	Low
NB-1771	Neuroblastoma	**0.004**	1.4	>4	0.75	0.075	Low	Low	Low
NB-EBc1	Neuroblastoma	**0.016**	1.4	>4	0.72	0.579	Low	Low	Low
NB-1643	Neuroblastoma	0.152	1.1	>4	0.74	0.075	Low	Low	Low
OS-1	Osteosarcoma	<**0.001**	1.2	>4	0.65	<0.001	Low	Low	Low
OS-2	Osteosarcoma	0.698	1.1	>4	0.9	0.529	Low	Low	Low
OS-17	Osteosarcoma	**0.029**	1.2	>4	0.71	0.105	Low	Low	Low
OS-9	Osteosarcoma	0.201	1.3	>4	0.71	0.028	Low	Low	Low
OS-33	Osteosarcoma	0.127	1	>4	0.74	0.247	Low	Low	Low

Xenograft Line	Histology	P-value	EFS T/C	Median Final RTV	Tumor Volume T/C	P-value	T/C Activity	EFS Activity	Response Activity
OS-31	Osteosarcoma	<**0.001**	1.2	>4	0.63	0.003	Low	Low	Low
ALL-2	ALL B-precursor	0.818	1.5	>25	.			Low	Int
ALL-3	ALL B-precursor	**0.041**	11.2	>25	.			Int	Int
ALL-4	ALL B-precursor	0.371	2.1	>25	.			Low	Int
ALL-7	ALL B-precursor	0.707	1.4	>25	.			Low	Low
ALL-8	ALL T-cell	**0.001**	4.7	>25	.			Int	Int
ALL-19	ALL B-precursor	0.703	3.4	>25	.			Low	Int